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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/058,323	04/09/98	HOUWEN B	10690/101683

EXAMINER	
GABEL, G	
ART UNIT	**PAPER NUMBER**
1641	12

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DATE MAILED: 01/17/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No.	Applicant(s)	
	09/058,323	HOUWEN ET AL.	
	Examiner	Art Unit	
	Gailene R. Gabel	1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).

Status

1)☒ Responsive to communication(s) filed on *17 October 2000* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-13* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a)☐ All b)☐ Some * c)☐ None of the CERTIFIED copies of the priority documents have been:

1.☐ received.

2.☐ received in Application No. (Series Code / Serial Number) _____ .

3.☐ received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. & 119(e).

Attachment(s)

14) ☐ Notice of References Cited (PTO-892)
15) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
16) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
17) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
18) ☐ Notice of Informal Patent Application (PTO-152)
19) ☐ Other: .

DETAILED ACTION

Amendment Entry

1. Applicants' amendment and response filed 10/17/2001 in Paper No. 11 is acknowledged and has been entered. Claims 1, 3-4, and 10 have been amended. Claim 13 has been added. Currently, claims 1-13 are pending and under examination.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

2. Claims 4 and 13 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 4, steps (i) and (ii) remain vague and confusing in relation to the preamble of the claim and inconsistent with the other claims.

In claim 4, steps (i) and (ii), the following language is suggested but not required to assist Applicants in clarifying the claim consistent with the preamble as well as other claims of the instant invention.

--(i) admixing a first reagent fluid of hypotonic osmolarity containing a buffer for maintaining a pH within an acidic range to the hematologic sample after step (i), thereby

raising the permeability of the erythroblast cell membranes to the nucleotide fluorescent dye; and

(ii) admixing (thereto) a second reagent fluid containing a buffer for neutralizing the first reagent fluid in the hematologic sample mixture and adjusting the pH of the mixture to an alkaline range of 5.0 – 11.0, thereby creating a condition suitable for staining the nuclei of the erythroblasts and an osmolarity compensating agent for adjusting the osmolarity to about 300 – 1000 mOsm/Kg H2O, thereby creating a condition suitable for retaining the shape and integrity of the leucocytes in the hematologic sample.-- (see pages 11 and 12 of the specification).

Claim 13 is vague and indefinite in reciting "wherein the integrity is about 400 mOsm/Kg.H2O to about 600 mOsm/Kg.H2O" because it is unclear how the term "integrity" and the range set forth in the claim interrelate. Do Applicants intend to recite an osmolarity range, for example.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of

the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103© and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

3. Claims 1-12 and also 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Loken et al. (US 5,047,321) in view of Kim et al. (US 5,559,037) and Inami et al. (US 5,298,426) for reason of record.

Response to Arguments

4. Applicants argue that the cited references fail to teach each element of the Applicants' claims and further fail to provide the requisite suggestion to provide the teaching of the instant invention. Specifically, Applicants contend that Loken fails to teach increasing permeability of cytoplasm of specific nucleated cells, in this case the erythroblasts, and that erythroblasts cannot be clearly discriminated from other cells let alone using a two-dimensional distribution chart. Applicants further contend that Kim fails to teach a dye or fluorescent labeled antibody to stain leucocytes, teaches discriminating erythroblasts by detecting three parameters rather than two as taught by the instant invention, and fails to address the deficiencies of Loken. Lastly, Applicants

contend that Inami fails to disclose use of two fluorescent light parameters and fails to disclose staining leucocytes with a fluorescent labeled antibody as required in claim 1.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). In response to applicant's argument that there is no suggestion to combine the three cited references, the test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference; nor is it that the claimed invention must be expressly suggested in any one or all of the references. Rather, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981).

In this case, Loken uses flow cytometry to analyze, measure, and discriminate fluorescence intensity and light scatter between each cell population in whole blood samples. Loken uses nucleotide fluorescent dyes to stain nuclei of nucleated cells to dyes independently and differentially assess different characteristics of nucleated cells and at least one fluorescent labeled antibody to label cell surface antigens in the sample.

Also, Kim teaches flow cytometric analysis of erythroblasts and leucocytes and incorporates a diluent that lyses erythroblasts to allow exposure of nuclei while

preserving the integrity and shape of leucocytes. Kim teaches exposing the nuclei of erythroblasts to nucleotide fluorescent dyes including Propidium iodide and ethidium bromide and constructs three-dimensional plots of qualified intensity signals of fluorescence and scattered light from detected signals to differentiate and quantitate erythroblasts and leucocytes.

Lastly, Inami uses flow cytometric analysis in differentiating erythroblasts from leucocytes. Inami adds to a blood sample, 1) hypotonic fluorescent dye solution to enable diffusion of nucleotide fluorescent dye into erythroblasts to stain their nuclei then a buffer for maintaining the pH in the acidic range 2) a solution comprising a buffer that neutralizes the acidic pH in the mixture and an osmolarity adjusting agent for adjusting the osmolarity to retain the shape and integrity of leucocytes. Inami uses different dyes and concentrations for differentiating leucocytes and erythroblasts, including propidium iodide and ethidium bromide; specifically, Inami teaches the concentration of there dyes to fall within the range of 0.003 mg/L to 10 mg/L (2.5 µg/ml to 100µg/ ml) in order to achieve optimum results and suggests incorporating leucocyte staining dyes in the first fluid. Stained cells are measured and anaylyzed using flow cytometry, erythroblasts are separated from other nucleated cell groups on the resulting two-dimensional plot where erythroblasts are counted.

It would have been obvious to one of ordinary skill in the art at the time of the invention to combine teachings of Kim and Inami into the method of Loken in differentiating between nucleated cells using nucleic acid dyes and fluorescent labeled

antibodies because all three cited references utilize flow cytometry in differentiating stained/labeled nucleated cellular populations and Inami specifically suggested using his method in combination with specific nuclear dyes in order to allow better differentiation between erythroblasts and leucocytes. One of ordinary skill in the art at the time of the instant invention would have been motivated to combine the teachings of Kim and Inami with the method of Loken in differentiating nucleated hematopoietic cell populations because it allows for simultaneous differentiation between desired populations, in this case, erythroblasts from leucocyte populations. In as far the teaching of two-dimensional distribution chart, two-dimensional plotting and statistical distribution of populations as effected by characteristic morphology or fluorescence intensities in combination with flow cytometry, i.e. scattergrams, is conventional and well known in the art.

5. Applicant's arguments filed 10/17/00 have been fully considered but they are not persuasive. Accordingly, no claims are allowed.

6. Applicant's amendment necessitated the new ground of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gail Gabel whose telephone number is (703) 305-0807. The examiner can normally be reached on Monday to Thursday from 7:00 AM to 4:30 PM. The examiner can also be reached on alternate Fridays from 7:00 AM to 3:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le, can be reached on (703) 308-4027. The fax phone number for the organization where this application or proceeding is assigned is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Gail Gabel 1/10/01
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